FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
A33002-PCT USA	09/486094

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR98/01814	18 August 1998	20 August 1997

TITLE OF INVENTION

GENE CODING FOR ANDROCTONINE, VECTOR CONTAINING SAME AND TRANSFORMED DISEASE-RESISTANT PLANTS OBTAINED

APPLICANT(S) FOR DO/EO/US

FREYSSINET, Georges; DEROSE, Richard; and HOFFMANN, Jules

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Form PCT/RO/101; Forms PCT/IPEA/409/416, Forms PCT/ISA/210; Forms PCT/IB/304/308 and a check in the amount of $1,312.

Express Mail No. EJ621295275US
Date of Deposit: 18 February 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/486094	PCT/FR98/01814	A33002-PCT USA

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$970.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	39 - 20 =	19	x $18.00	**$342.00**	
Independent claims	1 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,312.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).**			☐	**$0.00**	
SUBTOTAL =				**$1,312.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,312.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,312.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$1,312.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **02-4377** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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35,263
REGISTRATION NUMBER

18 February 2000
DATE

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	FREYSSINET, Georges et al.
Serial No.	:	To be assigned
Filed	:	18 August 1998
For	:	GENE CODING FOR ANDROCTONINE, VECTOR CONTAINING SAME AND TRANSFORMED DISEASE RESISTANT PLANTS OBTAINED

Express Mail Mailing No. EJ621295275US

PRELIMINARY AMENDMENT

Assistant Commissioner of Patent
Box PCT
Washington, D.C., 20231

Sir or Madam:

Prior to examination of the above-identified application, please amend the claims as follows:

IN THE CLAIMS:

Claim 3, Line 2:	please delete "either of claims 1 and 2" and substitute therefor --claim 1--.
Claim 4, Lines 1-2:	please delete "one of claims 1 to 3" and substitute therefor --claim 1--.
Claim 7, Lines 1-2:	please delete "one of claims 4 to 6" and substitute therefor --claim 4--.
Claim 9, Lines 1-2:	please delete "one of claims 1 to 8" and substitute therefor --claim 1--.
Claim 11, Lines 5-6:	please delete "one of claims 1 to 9" and substitute therefor --claim 1--.

Claim 18, Line 4: please delete "claims 11 to 16" and substitute therefor --claim 11--.

Claim 19, Line 8: please delete "claims 1 to 17" and substitute therefor --claim 1--.

Claim 21, Lines 1-2: please delete "either of claims 19 and 20" and substitute therefor --claim 19--.

Claim 22, Line 4: please delete "claims 19 to 21" and substitute therefor --claim 19--.

Claim 23, Line 4: please delete "claims 19 to 21" and substitute therefor --claim 19--.

Claim 24, Line 3: please delete "the vector according to claim 22" and substitute therefor -- a vector--.

Claim 25, Lines 1-2: please delete "either of claims 23 and 24" and substitute therefor --claim 23--.

Claim 28, Lines 3-4: please delete "one of claims 19 to 21" and substitute therefor --claim 19--.

Claim 33, Lines 1-2: please delete "one of claims 30 to 32" and substitute therefor --claim 30--.

Claim 34, Lines 1-2: please delete "one of claims 30 to 33" and substitute therefor --claim 30--.

Claim 35, Lines 1-2: please delete "one of claims 30 to 34" and substitute therefor --claim 30--.

Claim 36, Lines 2-3: please delete "one of claims 30 to 34, or obtained by the process according to claim 27" and substitute therefor --claim 30--.

Claim 39, Line 1: please delete "the".

Claim 39, Line 2: please delete "defined according to one of claims 1 to 18".

Claim 39, Line 4: please delete "either of claims 28 and 29" and substitute therefor --claim 28--.

REMARKS

The claims have been amended to remove multiple dependencies. Favorable consideration and allowance of all pending claims is respectfully requested.

Respectfully submitted,

BAKER BOTTS LLP

Dated: February 18, 2000

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Gene coding for androctonine, vector containing it and disease-resistant transformed plants obtained

The present invention relates to a DNA sequence coding for androctonine, to a vector containing it for the transformation of a host organism and to the process for transforming the said organism.

The invention relates more particularly to the transformation of plant cells and plants and to the androctonine produced by the transformed plants, giving them resistance to diseases, in particular diseases of fungal origin.

There is today an increasing need to make plants resistant to diseases, in particular fungal diseases, in order to reduce, or even avoid altogether, the need for treatments with antifungal protection products, in order to protect the environment. One means of increasing this disease-resistance consists in transforming the plants so that they produce substances capable of defending them against these diseases.

Various substances of natural origin are known, in particular peptides, which have bactericidal or fungicidal properties, especially against the fungi responsible for plant diseases. However, the problem consists in finding such substances which not only can be produced by transformed plants, but also can conserve their bactericidal or fungicidal properties and confer these properties to the said plants. For the

purposes of the present invention, the terms bactericidal and fungicidal are understood to refer both to the actual bactericidal or fungicidal properties and to the bacteriostat or fungistat properties.

Androctonines are peptides produced by scorpions, in particular from the species *Androctonus australis*. An androctonine and its preparation by chemical synthesis are described by Ehret-Sabatier et al., along with its *in vitro* antifungal and antibacterial properties.

The androctonine genes have now been identified, and it has also been found that they can be inserted into a host organism, in particular a plant, in order to express an androctonine, both for the preparation and isolation of this androctonine and to give the said host organism properties of resistance to fungal diseases and to diseases of bacterial origin, thereby providing a particularly advantageous solution to the problem outlined above.

The subject of the invention is thus, firstly, a nucleic acid fragment coding for an androctonine, a chimeric gene comprising the said fragment coding for an androctonine and heterologous regulation elements in positions 5' and 3' which can function in a host organism, in particular in plants, and a vector for transforming host organisms containing this chimeric gene, and the host organism transformed.

The invention also relates to a transformed plant cell containing at least one nucleic acid fragment coding for an androctonine, and to a disease-resistant plant containing the said cell, in particular a plant regenerated from this cell. Lastly, the invention relates to a process for cultivating transformed plants according to the invention.

According to the invention, the term androctonine is understood to refer to any peptide which can be produced by and isolated from scorpions, in particular from the species *Androctonus australis*, these peptides comprising at least 20 amino acids, preferably at least 25, and 4 cysteine residues which form disulphide bridges between themselves.

Advantageously, the androctonine essentially comprises the peptide sequence of general formula (I) below:

Xaa-Cys-Xab-Cys-Xac-Cys-Xad-Cys-Xae

(I)

in which

Xaa represents a peptide residue comprising at least 1 amino acid,

Xab represents a peptide residue of 5 amino acids,

Xac represents a peptide residue of 5 amino acids,

Xad represents a peptide residue of 3 amino acids, and

Xae represents a peptide residue comprising at least 1 amino acid.

Advantageously, Xab and/or Xad and/or Xae comprise at least one basic amino acid, preferably 1.

According to the invention, the term basic amino acids is understood to refer to amino acids chosen from lysine, asparagine and homoasparagine.

Preferably,

Xaa represents the peptide sequence Xaa'-Val, in which Xaa' represents NH_2 or a peptide residue comprising at least 1 amino acid, and/or

Xab represents the peptide sequence -Arg-Xab'-Ile, in which Xab' represents a peptide residue of 3 amino acids, and/or

Xac represents the peptide sequence -Arg-Xac'-Gly-, in which Xac' represents a peptide residue of 3 amino acids, and/or

Xad represents the peptide sequence -Tyr-Xad'-Lys, in which Xad' represents a peptide residue of 1 amino acid, and/or

Xae represents the peptide sequence -Thr-Xae', in which Xae' represents COOH or a peptide residue comprising at least 1 amino acid.

Preferably,

Xaa' represents the peptide sequence Arg-Ser-, and/or

Xab' represents the peptide sequence -Gln-Ile-Lys-, and/or

Xac' represents the peptide sequence -Arg-Arg-Gly-, and/or

Xad' represents the peptide residue -Tyr-, and/or

Xae' represents the peptide sequence -Asn-Arg-Pro-Tyr.

According to a preferred embodiment of the

invention, androctonine is represented by the peptide sequence of 25 amino acids described by the sequence identifier No. 1 (SEQ ID NO. 1) and the homologous peptide sequences.

The term homologous peptide sequences is understood to refer to any equivalent sequence comprising at least 65% homology with the sequence represented by the sequence identifier No. 1, it being understood that the 4 cysteine residues and the number of amino acids separating them remain identical, certain amino acids being replaced with different but equivalent amino acids on sites which do not induce a substantial change in the antifungal or antibacterial activity of the said homologous sequence. Preferably, the homologous sequences comprise at least 75% homology, more preferably at least 85% homology and even more preferably 90% homology.

The NH_2-terminal residue of androctonine can exhibit a post-translational modification, for example an acetylation, while the C-terminal residue can exhibit a post-translational modification, for example an amidation.

The expression peptide sequence essentially comprising the peptide sequence of general formula (I) is understood to refer not only to the sequences defined above, but also to such sequences comprising, at one or other of their ends or at both ends, peptide residues required for their expression and targeting in

a host organism, in particular a plant cell or plant.

This in particular concerns a "peptide-androctonine" or "androctonine-peptide", advantageously "peptide-androctonine", fusion peptide whose cleavage by the enzymatic systems of the plant cells allows the release of the androctonine defined above. The peptide fused to androctonine can be a signal peptide or a transit peptide which allows the production of androctonine to be controlled and oriented specifically in one part of the host organism, in particular of the plant cell or plant, such as, for example, the cytoplasm or the cell membrane, or in the case of plants, in a specific type of cell or tissue compartment or in the extracelluar matrix.

According to one embodiment, the transit peptide can be a chloroplast-addressing signal or a mitochondrion-addressing signal, which is then cleaved off in the chloroplasts or the mitochondria.

According to another embodiment of the invention, the signal peptide can be an N-terminal signal or "prepeptide", optionally in combination with a signal responsible for retaining the protein in the endoplasmic reticulum, or a vacuole-addressing peptide or "propeptide". The endoplasmic reticulum is the site at which maturation operations on the protein produced, such as, for example, cleavage of the signal peptide, are undertaken by the "cell machinery".

The transit peptides can be single or double,

and, in this case, optionally separated by an intermediate sequence, i.e. one comprising, in the direction of transcription, a sequence coding for a transit peptide of a plant gene which codes for a plastid localization enzyme, a portion of sequence of the N-terminal mature portion of a plant gene coding for a plastid localization enzyme, and then a sequence coding for a second transit peptide of a plant gene coding for a plastid localization enzyme, as described in patent application EP 0,508,909.

As transit peptide which is useful according to the invention, mention may be made in particular of the signal peptide of the tobacco PR-1α gene (WO 95/19443), represented with its coding sequence by the sequence identifier No. 2 (SEQ ID NO. 2) and fused to androctonine by the sequence identifier No. 3 (SEQ ID NO. 3), in particular corresponding to the fusion protein corresponding to bases 12 to 176 of this sequence, in particular when the androctonine is produced by plant cells or plants, or the precursor of Mat α1 factor when the androctonine is produced in yeasts.

The present invention thus relates, firstly, to a nucleic acid fragment, in particular a DNA fragment, coding for the androctonine defined above. According to the invention, this can be a fragment isolated from *Androctonus australis*, or alternatively a derived fragment, adapted for the expression of

androctonine in the host organism in which the peptide will be expressed. The nucleic acid fragment can be obtained according to the standard methods for isolation and purification, or alternatively by synthesis according to the usual techniques of successive hybridizations of synthetic oligonucleotides. These techniques are described in particular by Ausubel et al.

According to the present invention, the expression "nucleic acid fragment" is understood to refer to a nucleotide sequence which can be of DNA or RNA type, preferably of DNA type, in particular cDNA, especially of double-stranded type.

According to one embodiment of the invention, the nucleic acid fragment coding for androctonine is the DNA sequence described by the sequence identifier No. 1 (SEQ ID NO. 1), a homologous sequence or a sequence complementary to the said sequence, more particularly the coding portion of this SEQ ID NO. 1, corresponding to bases 1 to 75.

According to the invention, the term "homologous" is understood to refer to a nucleic acid fragment having one or more sequence modifications when compared with the nucleotide sequence described by the sequence identifier No. 1 coding for androctonine. These modifications can be obtained according to the usual mutation techniques, or alternatively by selecting the synthetic oligonucleotides used in the

preparation of the said sequence by hybridization. With regard to multiple combinations of nucleic acids which can lead to the expression of the same amino acid, the differences between the reference sequence described by the sequence identifier No. 1 and the homologous sequence can be considerable, and all the more so when it concerns a DNA fragment less than 100 nucleic acids in size, which can be produced by synthesis. Advantageously, the degree of homology will be at least 70% relative to the reference sequence, preferably at least 80% and more preferably at least 90%. These modifications are generally neutral, i.e. they do not affect the primary sequence of the resulting androctonine.

The present invention also relates to a chimeric gene (or expression cassette) comprising a coding sequence and heterologous regulation elements in positions 5' and 3' which can function in a host organism, in particular plant cells or plants, these elements being functionally linked to the said coding sequence, the said coding sequence comprising at least one DNA fragment coding for androctonine as defined above (including the "peptide-androctonine" or "androctonine-peptide" fusion peptide).

The term host organism is understood to refer to any lower-order or higher-order monocellular or multicellular organism into which the chimeric gene according to the invention can be introduced, for the

production of androctonine. Such organisms are, in particular, bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyces* or *Kluyveromyces, Pichia*, fungi, in particular *Aspergillus*, a baculovirus, or, preferably, plant cells and plants.

According to the invention, the term "plant cell" is understood to refer to any plant-derived cell which can constitute undifferentiated tissues such as calli, differentiated tissues such as embryos, plant portions, plants or seeds.

According to the invention, the term "plant" is understood to refer to any differentiated multicellular organism capable of photosynthesis, in particular monocotyledons or dicotyledons, more particularly crop plants which may or may not be intended for human or animal consumption, such as corn, wheat, rapeseed, soybean, rice, sugar cane, beetroot, tobacco, cotton, etc.

The regulation elements required for the expression of the DNA fragment coding for androctonine are well known to those skilled in the art as a function of the host organism. They comprise in particular promoter sequences, transcription activators, transit peptides and termination sequences, including start and stop codons. The means and methods for identifying and selecting the regulation elements are well known to those skilled in the art.

For the transformation of microorganisms such as yeasts or bacteria, the regulation elements are well known to those skilled in the art and comprise, in particular, promoter sequences, transcription activators, transit peptides, termination sequences and start and stop codons.

In order to direct the expression and secretion of the peptide in the yeast culture medium, a DNA fragment coding for heliomycin is incorporated into a shuttle vector which comprises the following elements:

- markers which allow the transformants to be selected,
- a nucleic acid sequence which allows replication (origin of replication) of the plasmid in the yeast,
- a nucleic acid sequence which allows replication (origin of replication) of the plasmid in *E. coli*,
- an expression cassette consisting of

(1) a promoter regulation sequence,

(2) a sequence coding for a signal peptide (or prepeptide) combined with an addressing peptide (or propeptide),

(3) a polyadenylation or terminator regulation sequence.

These elements have been described in several publications, including Reichhart et al., 1992, Invert. Reprod. Dev., 21, pp. 15-24 and Michaut et al., 1996, FEBS Letters, 395, pp. 6-10.

Preferably, yeasts from the species *S.*

cerevisiae are transformed with the expression plasmid by the lithium acetate method (Ito et al., 1993, J. Bacteriol, 153, pp. 163-168).

The invention relates more particularly to the transformation of plants. As promoter regulation sequence in plants, it is possible to use any promoter sequence of a gene which is naturally expressed in plants, in particular a promoter of bacterial, viral or plant origin such as, for example, that of a gene for the small subunit of ribulose biscarboxylase/oxygenase (RuBisCO) or of a plant virus gene such as, for example, that of cauliflower mosaic virus (CAMV 19S or 35S), or a promoter which can be induced by pathogens such as tobacco PR-1a, it being possible to use any suitable known promoter. Preferably, use is made of a promoter regulation sequence which favours the overexpression of the coding sequence in a constitutive manner or induced by the attack of a pathogen, such as, for example, that comprising at least one histone promoter as described in patent application EP 0,507,698.

According to the invention, it is also possible to use, in combination with the promoter regulation sequence, other regulation sequences which are located between the promoter and the coding sequence, such as transcription activators ("enhancers"), such as, for example, the tobacco mosaic virus (TMV) translation activator described in patent

application WO 87/07644, or the tobacco etch virus (TEV) translation activator described by Carrington & Freed.

As polyadenylation or terminator regulation sequence, it is possible to use any corresponding sequence of bacterial origin, such as, for example, the nos terminator of *Agrobacterium tumefaciens*, or alternatively of plant origin, such as, for example, a histone terminator as described in patent application EP 0,633,317.

According to the present invention, the chimeric gene can also be combined with a selection marker adapted to the transformed host organism. Such selection markers are well known to those skilled in the art. Such a marker may be an antibiotic-resistance gene or alternatively a herbicide-tolerance gene for plants.

The present invention also relates to a cloning or expression vector for the transformation of a host organism containing at least one chimeric gene as defined above. Besides the above chimeric gene, this vector comprises at least one origin of replication and, where appropriate, a suitable selection marker. This vector can consist of a plasmid, a cosmid, a bacteriophage or a virus, which are transformed by introducing the chimeric gene according to the invention. Depending on the host organism to be transformed, such transformation vectors are well known

to those skilled in the art and are widely described in the literature.

For the transformation of plant cells or plants, such a vector is, in particular, a virus which can be used for the transformation of the plants developed and also containing its own replication and expression elements. Preferably, the vector for transforming the plant cells or plants according to the invention is a plasmid.

The subject of the invention is also a process for transforming host organisms, in particular plant cells, by incorporating at least one nucleic acid fragment or one chimeric gene as defined above, it being possible for this transformation to be obtained by any suitable known means, which is amply described in the specialized literature, and in particular the references cited in the present application, more particularly by means of the vector according to the invention.

One series of methods consists in bombarding cells, protoplasts or tissues with particles to which the DNA sequences are attached. Another series of methods consists in using, as a means of transfer into the plant, a chimeric gene inserted into a Ti plasmid of *Agrobacterium tumefaciens* or an Ri plasmid of *Agrobacterium rhizogenes*.

Other methods can be used, such as microinjection or electroporation, or alternatively

direct precipitation using PEG.

A person skilled in the art will select the appropriate method as a function of the nature of the host organism, in particular the plant cell or plant.

The subject of the present invention is also transformed host organisms, in particular plant cells or plants, containing an effective amount of a chimeric gene comprising a sequence coding for the androctonine defined above.

The subject of the present invention is also plants containing transformed cells, in particular plants regenerated from the transformed cells. The regeneration is obtained by any suitable process which depends on the nature of the species, as described, for example, in the above references.

For the processes for transforming plant cells and for regenerating plants, mention will be made in particular of the following patents and patent applications: US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267,159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174, EP 486 233, EP 486 234, EP 539 563, EP 674 725, WO 91/02071 and WO 95/06128.

The subject of the present invention is also

the transformed plants obtained from the cultivating and/or crossing of the above regenerated plants, as well as the seeds of transformed plants.

The plants thus transformed are resistant to certain diseases, in particular to certain fungal or bacterial diseases. Consequently, the DNA sequence coding for androctonine can be inserted with the main aim of producing plants that are resistant to the said diseases, since androctonine is effective against fungal diseases such as those caused by *Cercospora*, in particular *Cercospora beticola*, *Cladosporium*, in particular *Cladosporium herbarum*, *Fusarium*, in particular *Fusarium culmorum* or *Fusarium graminearum*, or by *Phytophthora*, in particular *Phytophthora cinnamomi*.

The chimeric gene may also advantageously be combined with at least one selection marker, such as one or more herbicide-tolerance genes.

The DNA sequence coding for androctonine can also be inserted as a selection marker during the transformation of plants with other sequences coding for other peptides or proteins of interest, such as, for example, herbicide-tolerance genes.

Such herbicide-tolerance genes are well known to those skilled in the art and are described in particular in patent applications EP 115,673, WO 87/04181, EP 337,899, WO 96/38567 or WO 97/04103.

Needless to say, the transformed cells and

unpublished patent application FR 97/09115 filed on 24 July 1997.

Lastly, the present invention relates to a process for cultivating transformed plants according to the invention, the process consisting in planting the seeds of the said transformed plants in an area of a cultivation environment, in particular a field, which is suitable for cultivating the said plants, in applying an agrochemical composition to the said area, without substantially affecting the said transformed seeds or plants, and then in harvesting the plants cultivated when they reach the desired maturity, and optionally in separating the seeds from the harvested plants.

According to the invention, the term agrochemical composition is understood to refer to any agrochemical composition comprising at least one active product having either herbicidal, fungicidal, bactericidal, virucidal or insecticidal activity.

According to a preferred embodiment of the cultivation process according to the invention, the agrochemical composition comprises at least one active product having at least a fungicidal and/or bactericidal activity, more preferably having an activity complementary to that of the androctonine produced by the transformed plants according to the invention.

According to the invention, the expression

product having activity complementary to that of androctonine is understood to refer to a product having a complementary spectrum of activity, i.e. a product which will be active against attacks by androctonine-insensitive contaminants (fungi, bacteria or viruses), or alternatively a product whose spectrum of activity totally or partially covers that of androctonine, and whose dose of application will be substantially reduced on account of the presence of the androctonine produced by the transformed plant.

Lastly, cultivation of the transformed host organisms allows the large-scale production of androctonine. The subject of the present invention is thus also a process for preparing androctonine, comprising the steps of cultivating the transformed host organism comprising a gene coding for androctonine as defined above in an appropriate cultivation environment, followed by the extraction and total or partial purification of the androctonine obtained.

The examples below make it possible to illustrate the invention, the preparation of the sequence coding for androctonine, the chimeric gene, the integration vector and the transformed plants. The attached Figures 1 to 5 describe schematic structures of certain plasmids prepared for the construction of the chimeric genes. In these figures, the various restriction sites are marked in *italics*.

Example 1: Construction of the chimeric genes

All the techniques used below are standard laboratory techniques. The detailed procedures for these techniques are described in particular in Ausubel et al.

pRPA-MD-P: Creation of a plasmid containing the signal peptide for the tobacco PR-1a gene.

The two complementary synthetic oligonucleotides Oligo 1 and Oligo 2 below are hybridized at 65°C for 5 minutes and then by slowly decreasing the temperature to 30°C over 30 min.

Oligo 1: 5' GCGTCGACGC GATGGGTTTC GTGCTTTTCT CTCAGCTTCC
ATCTTTCCTT CTTGTGTCTA CTCTTCTTCT TTTCC 3'

Oligo 2: 5' TCGCCGGCAC GGCAAGAGTA AGAGATCACA AGGAAAAGAA
GAAGAGTAGA CACAAGAAGG AAAGATGGAA GC 3'

After hybridization between Oligo 1 and Oligo 2, the remaining single-stranded DNA serves as a matrix for the klenow fragment of *E. coli* polymerase 1 (under the standard conditions recommended by the manufacturer (New England Biolabs)) for the creation of the double-stranded oligonucleotide starting from the 3' end of each oligo. The double-stranded oligonucleotide obtained is then digested with the restriction enzymes *SacII* and *NaeI* and cloned in the plasmid pBS II SK(-) (Stratagene) digested with the

same restriction enzymes. A clone comprising the region coding for the signal peptide of the tobacco PR-1a gene (SEQ ID NO. 2) is thus obtained.

pRPA-PS-PR1a-andro: Creation of a sequence coding for androctonine fused to the PR-1a signal peptide without an untranscribed 3' region.

The two complementary synthetic oligonucleotide sequences Oligo 3 and Oligo 4 are hybridized according to the operating conditions described for pRPA-MD-P.

Oligo 3: 5' AGGTCCGTGT GCAGGCAGAT CAAGATCTGC AGGAGGAGGG GTGG 3'

Oligo 4: 5' CCGGATCCGT CGACACGTTC GCCTCGCCGA GCTCAGTATG GCCTGTTAGT GCACTTGTAG TAGCAACCAC CCCTCCTCCT GCAGATCTTG ATCTGCC 3'

After hybridization between Oligo 3 and Oligo 4, the remaining single-stranded DNA serves as a matrix for the klenow fragment of *E. coli* polymerase 1 (under the standard conditions recommended by the manufacturer (New England Biolabs)) for the creation of the double-stranded oligonucleotide starting from the 3' end of each oligo. This double-stranded oligonucleotide containing the portion coding for androctonine (SEQ ID NO. 1) is then cloned directly in the plasmid pRPA-MD-P, which was digested with the

restriction enzyme *NaeI*. The correct orientation of the clone obtained is verified by sequencing. A clone comprising the region coding for the PR-1a-androctonine fusion protein, located between the *NcoI* restriction site at the N-terminal end and the *ScaI*, *SacII* and *BamHI* restriction sites at the C-terminal end (SEQ ID NO. 3), is thus obtained.

pRPA-RD-238: Creation of an expression vector in plants comprising the sequence coding for the PR-1a androctonine fusion protein.

The plasmid pRTL-2 GUS, derived from the plasmid pUC-19, was obtained from Dr. Jim Carrington (Texas A&M University, not described). This plasmid, whose schematic structure is represented in Figure 1, contains the duplicated CaMV 35S promoter isolated from cauliflower mosaic virus (CaMV 2x35S promoter; Odell et al., 1985) which directs the expression of an RNA containing a 5' untranslated sequence of tobacco etch virus (TEV 5' UTR; Carrington and Freed, 1990), the *E. coli* β-glucuronidase gene (GUS; Jefferson et al., 1987) followed by the CaMV RNA 35S polyadenylation site (CaMV polyA; Odell et al., 1985).

The plasmid pRTL-2 GUS is digested with the restriction enzymes *NcoI* and *BamHI* and the main DNA fragment is purified. The plasmid pRPA-PS-PR1a-andro is digested with the restriction enzymes *NcoI* and *BamHI* and the small DNA fragment containing the region coding

for the PR-1a-androctonine fusion protein is purified. The two purified DNA fragments are then linked together in an expression cassette in the plants which synthesizes a PR-1a-androctonine fusion protein. The schematic structure of this expression cassette is represented in Figure 2. "PR-1a-androctonine" represents the region coding for the PR-1a-androctonine fusion protein of pRPA-RD-230. The androctonine is transported to the plant's extracelluar matrix by the action of the PR-1a peptide signal.

pRPA-RD-195: Creation of a plasmid containing a modified multiple cloning site.

The plasmid pRPA-RD-195 is a plasmid derived from pUC-19 which contains a modified multiple cloning site. The complementary synthetic oligonucleotides Oligo 5 and Oligo 6 below are hybridized and made double-stranded according to the procedure described for pRPA-MD-P.

Oligo 5: 5' AGGGCCCCCT AGGGTTTAAA CGGCCAGTCA GGCCGAATTC GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GACCTGCAGG CATGC 3'

Oligo 6: 5' CCCTGAACCA GGCTCGAGGG CGCGCCTTAA TTAAAAGCTT GCATGCCTGC AGGTCGACTC TAGAGG 3'

The double-stranded oligonucleotide obtained is then inserted into pUC-19, which was predigested

with the restriction enzymes *EcoRI* and *HindIII* and made blunt at the ends using the klenow fragment of *E. coli* DNA polymerase 1. A vector containing multiple cloning sites to facilitate the introduction of the expression cassettes into an *Agrobacterium tumefaciens* vector plasmid is obtained. The schematic structure of this multiple cloning site is represented in Figure 3.

pRPA-RD-233: Introduction of the PR-1a-androctonine expression cassette from pRPA-RD-230 into pRPA-RD-195.

The plasmid pRPA-RD-230 is digested with the restriction enzyme *HindIII*. The DNA fragment containing the PR-1a-androctonine expression cassette is purified. The purified fragment is then inserted into pRPA-RP-195, which was predigested with the restriction enzyme *HindIII* and dephosphorylated with calf intestinal phosphatase.

pRPA-RD-174: Plasmid derived from pRPA-BL-150A (EP 0,508,909) containing the bromoxynil-tolerance gene from pRPA-BL-237 (EP 0,508,909).

The bromoxynil-tolerance gene is isolated from pRPA-BL-237 by means of a PCR gene amplification. The fragment obtained has blunt ends, and is cloned in the pRPA-BL-150A *EcoRI* site, the ends of which were made blunt by the action of klenow polymerase under standard conditions. An *Agrobacterium tumefaciens* vector which contains the bromoxynil-tolerance gene

close to its right-hand end, a kanamycin-tolerance gene close to its left-hand end and a multiple cloning site between these two genes is obtained.

The schematic structure of pRPA-RD-174 is represented in Figure 4. In this figure, "nos" represents the polyadenylation site of *Agrobacterium tumefaciens* nopaline synthase (Bevan et al., 1983), "NOS pro" represents the *Agrobacterium tumefaciens* nopaline synthase promoter (Bevan et al., 1983), "NPT II" represents the neomycin phosphotransferase gene of the Tn5 transposon of *E. coli* (Rothstein et al., 1981), "35S pro" represents the 35S promoter isolated from cauliflower mosaic virus (Odell et al., 1985), "BRX" represents the nitrilase gene isolated from *K. ozaenae* (Stalker et al., 1988), "RB" and "LB" represent, respectively, the right-hand and left-hand ends of the sequence of an *Agrobacterium tumefaciens* Ti plasmid.

pRPA-RD-184: Addition of a new, unique restriction site into pRPA-RD-174.

The complementary synthetic oligonucleotides Oligo 7 and Oligo 8 below are hybridized and made double-stranded according to the procedure described for pRPA-MD-P.

Oligo 7: 5' CCGGCCAGTC AGGCCACACT TAATTAAGTT TAAACGCGGC
CCCGGCGCGC CTAGGTGTGT GCTCGAGGGC CCAACCTCAG
TACCTGGTTC AGG 3'

Oligo 8: 5' CCGGCCTGAA CCAGGTACTG AGGTTGGGCC CTCGAGCACA
CACCTAGGCG CGCCGGGGCC GCGTTTAAAC TTAATTAAGT
GTGGCCTGAC TGG 3'

The hybridized double-stranded oligonucleotide (96 base pairs) is purified after separation on agarose gel (3% Nusieve, FMC). The plasmid pRPA-RD-174 is digested with the restriction enzyme *XmaI* and the main DNA fragment is purified. The two DNA fragments obtained are then linked together.

A plasmid derived from pRPA-RD-174 is obtained, comprising other restriction sites between the bromoxynil-tolerance gene and the selection marker kanamycin gene.

The schematic structure of the plasmid pRPA-RD-184 is represented in Figure 5, in which the terms "nos", "NPT II", "NOS pro", "35S pro", "BRX gene", "RB" and "LB" have the same meanings as in Figure 4.

pRPA-RD-236: Creation of an *Agrobacterium tumefaciens* vector containing the gene construct coding for androctonine directed towards the extracellular matrix.

The plasmid pRPA-RD-233 is digested with the restriction enzymes *PmeI* and *AscI* and the DNA fragment containing the PR-1a-androctonine gene is purified. The plasmid pRPA-RD-184 is digested with the same restriction enzymes. The DNA fragment containing the PR-1a-androctonine expression cassette is then inserted

into pRPA-RD-184. An *Agrobacterium tumefaciens* vector containing the sequence coding for the PR-1a-androctonine fusion protein is thus obtained, which leads to the expression of androctonine in the plant's extracellular matrix.

Example 2: Tolerance to herbicides of transformed tobacco plants.

2.1- Transformation

The vector pRPA-RD-236 is introduced into the *Agrobacterium tumefaciens* strain EHA101 (Hood et al., 1987) carrying the cosmid pTVK291 (Komari et al., 1986). The transformation technique is based on the procedure by Horsh et al. (1985).

2.2- Regeneration

Regeneration of the tobacco plant PBD6 (obtained from SEITA France) from foliar explants is carried out on Murashige-Skoog (MS) base medium comprising 30 g/l of sucrose and 200 µg/ml of kanamycin. The foliar explants are taken from plants cultivated in a greenhouse or *in vitro* and regenerated according to the foliar disc technique (Horsh et al., 1985) in three successive steps: the first step comprises induction of the shoots on a medium supplemented with 30 g/l of sucrose containing 0.05 mg/l of naphthylacetic acid (NAA) and 2 mg/l of benzylaminopurine (BAP) for 2 weeks. The shoots formed during this step are then grown for 10 days by

cultivating on MS medium supplemented with 30 g/l of sucrose but containing no hormone. Next, the shoots which have grown are taken and cultivated on an MS rooting medium with half the content of salts, vitamins and sugar and containing no hormone. After about 2 weeks, the rooted shoots are placed in a greenhouse.

2.3- Tolerance to bromoxynil

Twenty transformed plants were regenerated and placed in a greenhouse for the construction of pRPA-RD-236. These plants were treated in the greenhouse, at the 5-leaf stage, with aqueous Pardner suspension corresponding to 0.2 kg of bromoxynil active material per hectare.

All the plants showing complete tolerance to bromoxynil are then used in various experiments which show that the expression of androctonine by the transformed plants makes them resistant to fungal attack.

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CLAIMS

1. Nucleic acid fragment, characterized in that it comprises a nucleic acid sequence coding for an androctonine.

2. Nucleic acid fragment according to claim 1, characterized in that it is a sequence of DNA.

3. Nucleic acid fragment according to either of claims 1 and 2, characterized in that the androctonine consists of a peptide which can be produced by and isolated from scorpions, in particular from the species *Androctonus australis*, the said peptide comprising at least 20 amino acids, preferably at least 25 amino acids, and 4 cysteine residues which form disulphide bridges between themselves.

4. Nucleic acid fragment according to one of claims 1 to 3, characterized in that the androctonine essentially comprises the peptide sequence of general formula (I) below

Xaa-Cys-Xab-Cys-Xac-Cys-Xad-Cys-Xae

(I)

in which

Xaa represents a peptide residue comprising at least 1 amino acid,

Xab represents a peptide residue of 5 amino acids,

Xac represents a peptide residue of 5 amino acids,

Xad represents a peptide residue of 3 amino acids, and

Xae represents a peptide residue comprising at least 1 amino acid.

5. Nucleic acid fragment according to claim 4, characterized in that Xab and/or Xad and/or Xae comprise at least one basic amino acid.

6. Nucleic acid fragment according to claim 5, characterized in that the basic amino acids are chosen from lysine, asparagine and homoasparagine.

7. Nucleic acid fragment according to one of claims 4 to 6, characterized in that
Xaa represents the peptide sequence Xaa'-Val, in which Xaa' represents NH_2 or a peptide residue comprising at least 1 amino acid, and/or
Xab represents the peptide sequence -Arg-Xab'-Ile, in which Xab' represents a peptide residue of 3 amino acids, and/or
Xac represents the peptide sequence -Arg-Xac'-Gly-, in which Xac' represents a peptide residue of 3 amino acids, and/or
Xad represents the peptide sequence -Tyr-Xad'-Lys, in which Xad' represents a peptide residue of 1 amino acid, and/or
Xae represents the peptide sequence -Thr-Xae', in which Xae' represents COOH or a peptide residue comprising at least 1 amino acid.

8. Nucleic acid fragment according to claim 7, characterized in that
Xaa' represents the peptide sequence -Arg-Ser-, and/or
Xab' represents the peptide sequence -Gln-Ile-Lys-, and/or

Xac' represents the peptide sequence -Arg-Arg-Gly-, and/or

Xad' represents the peptide residue -Tyr-, and/or

Xae' represents the peptide sequence -Asn-Arg-Pro-Tyr.

9. Nucleic acid fragment according to one of claims 1 to 8, characterized in that the androctonine is represented by the peptide sequence of 25 amino acids described by the sequence identifier No. 1 (SEQ ID NO. 1) and the homologous peptide sequences.

10. Nucleic acid fragment according to claim 9, characterized in that it is represented by the sequence identifier No. 1 (SEQ ID NO. 1), a homologous sequence or a sequence complementary to the said sequence, more particularly the coding portion of this SEQ ID NO. 1, corresponding to bases 1 to 75.

11. Nucleic acid fragment, characterized in that it comprises a nucleic acid sequence coding for a "peptide-androctonine" or "androctonine-peptide", advantageously "peptide-androctonine", fusion peptide, the androctonine being defined according to one of claims 1 to 9.

12. Nucleic acid fragment according to claim 11, characterized in that the peptide fused to androctonine is a signal peptide or a transit peptide.

13. Nucleic acid fragment according to claim 12, characterized in that the transit peptide is a chloroplast-addressing signal or a mitochondrion-addressing signal.

14. Nucleic acid fragment according to claim 12, characterized in that the signal peptide is an N-terminal signal or "prepeptide", optionally in combination with a signal responsible for retaining the protein in the endoplasmic reticulum, or a vacuole-addressing peptide or "propeptide".

15. Nucleic acid fragment according to claim 14, characterized in that the signal peptide is the signal peptide of the tobacco PR-1α gene.

16. Nucleic acid fragment according to claim 15, characterized in that the "peptide-androctonine" fusion peptide is represented by the sequence identifier No. 3 (SEQ ID NO. 3).

17. Nucleic acid fragment according to claim 16, characterized in that the coding sequence is represented by the sequence identifier No. 3 (SEQ ID NO. 3), a homologous sequence or a complementary sequence, more particularly the coding portion of this SEQ ID NO. 3, corresponding to bases 12 to 176 of this sequence.

18. "Peptide-androctonine" or "androctonine-peptide", preferably "peptide-androctonine", fusion protein, characterized in that it is defined according to claims 11 to 16.

19. Chimeric gene comprising a coding sequence and heterologous regulation elements in positions 5' and 3' which can function in a host organism, in particular plant cells or plants, these

elements being functionally linked to the said coding sequence, characterized in that the said coding sequence comprises at least one DNA fragment coding for androctonine as defined according to claims 1 to 17.

20. Chimeric gene according to claim 19, characterized in that the host organism is chosen from bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyces* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants.

21. Chimeric gene according to either of claims 19 and 20, characterized in that it is combined with a selection marker adapted to the transformed host organism.

22. Cloning or expression vector for the transformation of a host organism, characterized in that it comprises at least one chimeric gene as defined according to claims 19 to 21.

23. Process for transforming host organisms, in particular plant cells, by incorporating at least one nucleic acid fragment or one chimeric gene as defined in claims 19 to 21.

24. Process according to claim 23, characterized in that the chimeric gene is incorporated by means of the vector according to claim 22.

25. Process according to either of claims 23 and 24, characterized in that the host organism is chosen from bacteria, for example *E. coli*, yeasts, in

particular yeasts of the genera *Saccharomyces* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants.

26. Process according to claim 25, characterized in that the host organism is a plant cell.

27. Process according to claim 26, characterized in that plants are regenerated from transformed plant cells.

28. Transformed host organism, in particular plant cell or plant, characterized in that it comprises a chimeric gene defined according to one of claims 19 to 21.

29. Host organism according to claim 28, characterized in that it is chosen from bacteria, for example *E. coli*, yeasts, in particular yeasts of the genera *Saccharomyces* or *Kluyveromyces*, *Pichia*, fungi, in particular *Aspergillus*, a baculovirus, and plant cells and plants.

30. Plants, characterized in that they comprise transformed plant cells according to claim 29.

31. Plant according to claim 30, characterized in that it is regenerated from transformed plant cells.

32. Plant, characterized in that it is obtained from the cultivating and/or crossing of the regenerated plants according to claim 31.

33. Plant according to one of claims 30 to

32, characterized in that it is chosen from corn, wheat, rapeseed, soybean, rice, sugar cane, beetroot, tobacco and cotton.

34. Plant according to one of claims 30 to 33, characterized in that it is resistant to fungal diseases such as those caused by *Cercospora*, in particular *Cercospora beticola*, *Cladosporium*, in particular *Cladosporium herbarum*, *Fusarium*, in particular *Fusarium culmorum* or *Fusarium graminearum*, or by *Phytophthora*, in particular *Phytophthora cinnamomi*.

35. Plant seeds according to one of claims 30 to 34.

36. Process for cultivating transformed plants according to one of claims 30 to 34, or obtained by the process according to claim 27, the said process consisting in planting the seeds of the said transformed plants in an area of a cultivation environment, in particular a field, which is suitable for cultivating the said plants, in applying an agrochemical composition to the said area, without substantially affecting the said transformed seeds or plants, and then in harvesting the plants cultivated when they reach the desired maturity, and optionally in separating the seeds from the harvested plants.

37. Process according to claim 36, characterized in that the agrochemical composition comprises at least one active product having at least a

fungicidal and/or bactericidal activity.

38. Process according to claim 37, characterized in that the active product has an activity complementary to that of the androctonine produced by the transformed plants.

39. Process for preparing the androctonine defined according to one of claims 1 to 18, comprising the steps of cultivating the transformed host organism defined according to either of claims 28 and 29 in an appropriate cultivation environment, followed by the extraction and total or partial purification of the androctonine obtained.

WO 99/09189 PCT/FR98/01814

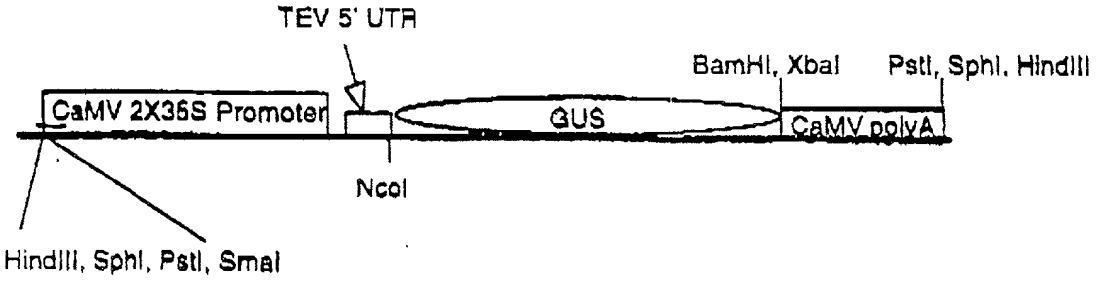

Fig. 1

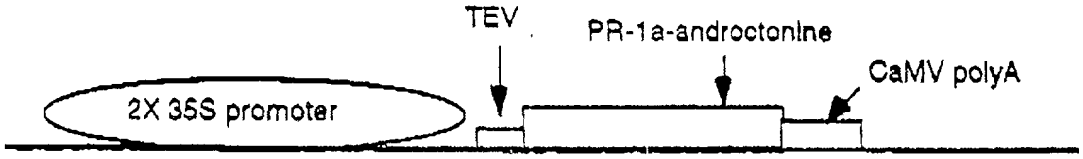

Fig. 2

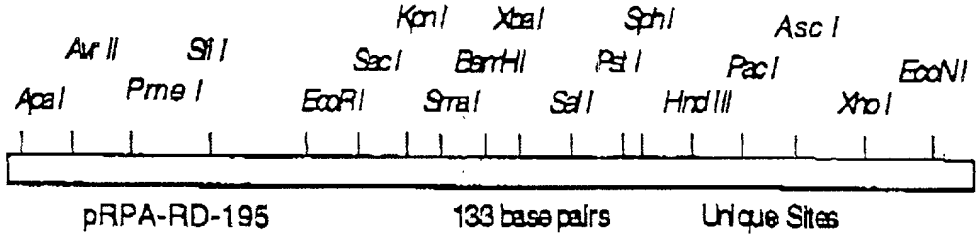

Fig. 3

2/2

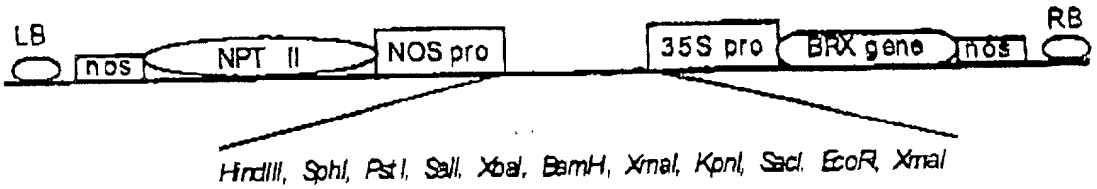
LB
nos
NPT II
NOS pro
35S pro
BRX gene
nos
RB
HindIII, SphI, PstI, SalI, XbaI, BamH, XmaI, KpnI, SacI, EcoR, XmaI

Fig. 4

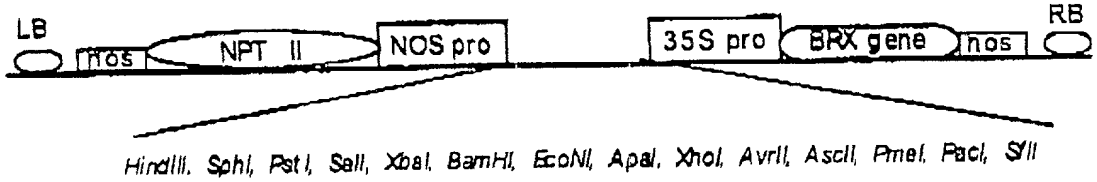
LB
nos
NPT II
NOS pro
35S pro
BRX gene
nos
RB
HindIII, SphI, PstI, SalI, XbaI, BamHI, EcoNI, ApaI, XhoI, AvrII, AscII, PmeI, PacI, SfiI

Fig. 5

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that: WE, GEORGES FREYSSINET, RICHARD DEROSE and JULES HOFFMANN My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

GENE CODING FOR ANDROCTONINE, VECTOR CONTAINING SAME AND DISEASE-RESISTANT TRANSFORMED PLANTS OBTAINED

This declaration is of the following type:

[] original
[] design
[X] national stage of PCT.
[] divisional
[] continuation
[] continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

(a) [ ] is attached hereto.
(b) [X] was filed on February 18, 2000 as Application Serial No. 09/486,094 and was amended on *(if applicable).*
(c) [X] was described and claimed in PCT International Application No.PCT/FR98/01814 filed August 18, 1998 on and was amended on *(if applicable).*

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[ ] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) [ ] no such applications have been filed.

(e) [X] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
France	FR 97/10632	August 20, 1997		[X] YES NO [ ]
				[ ] YES NO [ ]
				[ ] YES NO [ ]
ALL FOREIGN APPLICATION[S], IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
				[ ] YES NO [ ]
				[ ] YES NO [ ]
				[ ] YES NO [ ]

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Scheinfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	BAKER BOTTS L.L.P. (212) 705-5000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME FREYSSINET	FIRST NAME GEORGES	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY Saint Cyr Au Mont D'Or	STATE or FOREIGN COUNTRY FRANCE	COUNTRY OF CITIZENSHIP FRANCE	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 21 rue de Nervieux	CITY Saint Cyr Au Mont D'Or	STATE or COUNTRY France	ZIP CODE 69450
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME DEROSE	FIRST NAME RICHARD	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY Lyon	STATE or FOREIGN COUNTRY FRANCE	COUNTRY OF CITIZENSHIP FRANCE	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 216 rue de Saint Cyr	CITY Lyon	STATE or COUNTRY France	ZIP CODE 69009
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME HOFFMANN	FIRST NAME JULES	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY Strasbourg	STATE or FOREIGN COUNTRY FRANCE	COUNTRY OF CITIZENSHIP FRANCE	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 5 rue Closener	CITY Strasbourg	STATE or COUNTRY France	ZIP CODE 67000
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: RHONE-POULENC AGROCHIMIE

(B) STREET: 14-20 Rue Pierre BAIZET

(C) TOWN: LYONS

(E) COUNTRY: France

(F) POST CODE: 69009

(ii) TITLE OF THE INVENTION: Gene coding for androctonine, vector containing it and disease-resistant transformed plants obtained

(iii) NUMBER OF SEQUENCES: 11

(vi) COMPUTER-READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 110 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..75

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
AGG TCC GTG TGC AGG CAG ATC AAG ATC TGC AGG AGG AGG GGT GGT TGC  48
Arg Ser Val Cys Arg Gln Ile Lys Ile Cys Arg Arg Arg Gly Gly Cys
 1               5                  10                  15

TAC TAC AAG TGC ACT AAC AGG CCA TAC TGAGCTCGGC GAGGCGAACG       95
Tyr Tyr Lys Cys Thr Asn Arg Pro Tyr
            20                  25

TGTCGACGGA TCCGG                                              110
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 106 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 12..101

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
GCGTCGACGC C ATG GGT TTC GTG CTT TTC TCT CAG CTT CCA TCT TTC CTT 50
             Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu
               1               5                  10

CTT GTG TCT ACT CTT CTT CTT TTC CTT GTG ATC TCT CAC TCT TGC CGT  98
Leu Val Ser Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg
    15                  20                  25

GCC GGCGA                                                      106
Ala
 30
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 12..176

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
GCGTCGACGC C ATG GGT TTC GTG CTT TTC TCT CAG CTT CCA TCT TTC CTT   50
             Met Gly Phe Val Leu Phe Ser Gln Leu Pro Ser Phe Leu
               1               5                  10

CTT GTG TCT ACT CTT CTT CTT TTC CTT GTG ATC TCT CAC TCT TGC CGT   98
Leu Val Ser Thr Leu Leu Leu Phe Leu Val Ile Ser His Ser Cys Arg
     15                  20                  25

GCC AGG TCC GTG TGC AGG CAG ATC AAG ATC TGC AGG AGG AGG GGT GGT   146
Ala Arg Ser Val Cys Arg Gln Ile Lys Ile Cys Arg Arg Arg Gly Gly
 30                  35                  40                  45

TGC TAC TAC AAG TGC ACT AAC AGG CCA TAC TGAGCTCGGC GAGGCGAACG     196
Cys Tyr Tyr Lys Cys Thr Asn Arg Pro Tyr
                50                  55

TGTCGACGGA TCCGG                                                 211
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 75 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGTCGACGC GATGGGTTTC GTGCTTTTCT CTCAGCTTCC ATCTTTCCTT CTTGTGTCTA 60

CTCTTCTTCT TTTCC 75

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TCGCCGGCAC GGCAAGAGTA AGAGATCACA AGGAAAAGAA GAAGAGTAGA CACAAGAAGG 60

AAAGATGGAA GC 72

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGGTCCGTGT GCAGGCAGAT CAAGATCTGC AGGAGGAGGG GTGG 44

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCGGATCCGT CGACACGTTC GCCTCGCCGA GCTCAGTATG GCCTGTTAGT GCACTTGTAG 60

TAGCAACCAC CCCTCCTCGT GCAGATCTTG ATCTGCC 97

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGGGCCCCCT AGGGTTTAAA CGGCCAGTCA GGCCGAATTC GAGCTCGGTA CCCGGGGATC 60

CTCTAGAGTC GACCTGCAGG CATGC 85

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic

oligonucleotide 6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCCTGAACCA GGCTCGAGGG CGCGCCCTTAA TTAAAAGCTT GCATGCCTGC AGGTCGACTC 60

TAGAGG 66

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 7"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CCGGCCAGTC AGGCCACACT TAATTAAGTT TAAACGCGGC CCCGGCGCGC CTAGGTGTGT 60

GCTCGAGGGC CCAACCTCAG TACCTGGTTC AGG 93

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic oligonucleotide 8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCGGCCTGAA CCAGGTACTG AGGTTGGGCC CTCGAGCACA CACCTAGGCG CGCCGGGGCC 60

GCGTTTAAAC TTAATTAAGT GTGGCCTGAC TGG 93